Sulphoxide-directed Disulphide Bond-forming Reaction for the Synthesis of Cystine Peptides

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Disulphide bonds are formed at the sulphur atom of Cys(R) sulphoxides intermolecularly as well as intramolecularly, following liberation of the SH group from the co-reactant, Cys(R'), by a suitable acid.

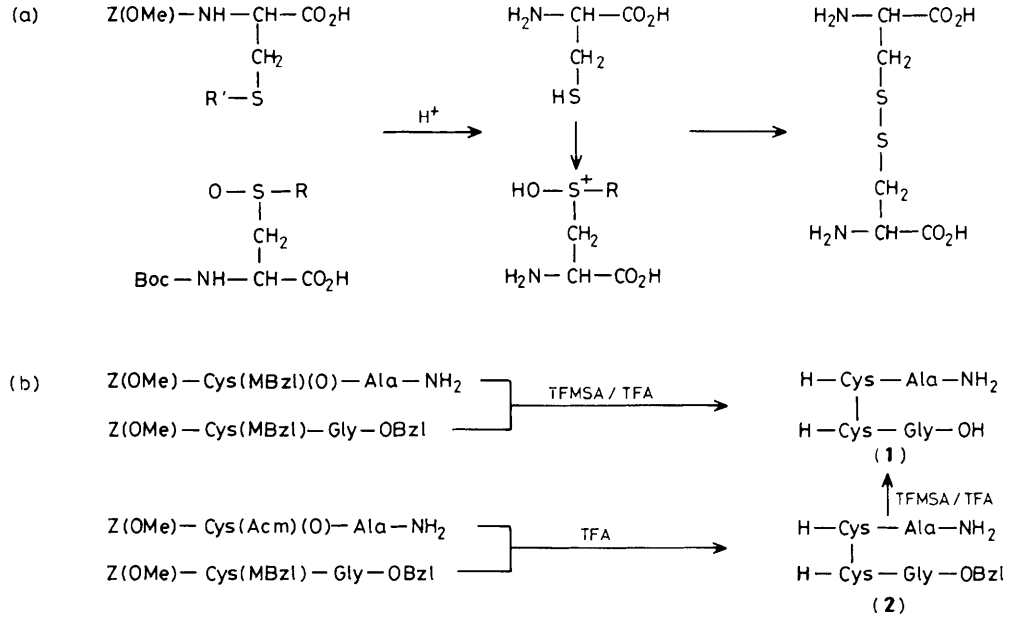
We report a new acid-catalysed disulphide bond-forming reaction, involving *S*-substituted cysteine sulphoxides, Cys(R)(O). In 1979, we reported that treatment of Z(OMe)-Cys(MBzl)(O)-OH with MSA (methanesulphonic acid) in the presence of a cation scavenger, anisole, afforded *p*-methoxyphenylcysteine as the major product.[1] Nucleophilic attack of the *para*-aromatic carbon atom of anisole at the sulphur atom of protonated Cys(MBzl)(O) seems to proceed in acid media. We have now found that the sulphur atom of cysteine plays the similar role, with generation of cystine in 86% yield when anisole is replaced by cysteine in the above treatment with MSA (0 °C; 60 min) and Me_2S (0.5 M) is used as an alternative scavenger (Table 1). The yield was improved when MSA was replaced by 1 M TFMSA (trifluoromethanesulphonic acid) in TFA (trifluoroacetic acid)[2] or by 1 M TMSOTf (trimethylsilyl trifluoromethanesulphonate) in TFA.[3] However, on treatment with TFA alone the yield was <10%. Cystine was obtained in fairly good yields when an equimolar mixture of Boc-Cys(Acm)(O)-OH[4] and cysteine was treated with the foregoing acids, including TFA. However, Z(OMe)-Cys(Ad)(O)-OH[5] gave poor results.

It was found further that this sulphoxide-directed disulphide

Table 1. Acid-catalysed disulphide bond-forming reaction between Cys(R)(O) and cysteine or Cys(R′).[a]

Sulphoxide	Acid	Product (%) Cysteine	Cys(MBzl)	Cys(Ad)
Cys(MBzl)(O)	TFA	9.1	7.3	2.8
	MSA	86.4	83.3	85.8
	1 M TFMSA in TFA	100.0	92.2	92.4
	1 M TMSOTf in TFA	100.0	84.7	84.8
Cys(Acm)(O)	TFA	97.9	85.3[b]	1.7
	1 M TFMSA in TFA	84.9	70.3	75.9
	1 M TMSOTf in TFA	80.0	84.3	86.3
Cys(Ad)(O)	1 M TFMSA in TFA	20.8	15.5	10.5

[a] Reactions were performed in the presence of Me_2S (0.5 M in TFA) in an ice-bath for 60 min. Abbreviations: MBzl = *p*-methoxybenzyl; Acm = acetamidomethyl; Ad = 1-adamantyl. [b] Reaction at 25 °C.



Scheme 1. Sulphoxide-directed bond-forming reactions. Z(OMe) = *p*-$MeOC_6H_4CH_2OC(=O)$–; Boc = $Bu^tOC(=O)$–.

bond-forming reaction proceeded, even if cysteine was replaced by a derivative Cys(R′) (R′ = acid cleavable *S*-protecting group, such as MBzl or Ad) (Scheme 1, a). Dimethyl sulphide is known to at as a soft base[6] in acid media, accelerating the cleavage reaction, and then regenerating the SH group. TFA treatment of an equimolar mixture of Boc-Cys(Acm)(O)-OH and Z(OMe)-Cys(MBzl)-OH (25 °C; 60 min) afforded cystine in 85% yield. Combinations of Cys(MBzl)(O) and Cys(R′) required stronger acid treatment in order to obtain cystine in satisfactory yields.

By using the above sulphoxide-directed disulphide bond-forming reaction, a model open-chain unsymmetrical cystine peptide was synthesized (Scheme 1, b). Treatment of an equimolar mixture of Z(OMe)-Cys(MBzl)(O)-Ala-NH_2 and Z(OMe)-Cys(MBzl)-Gly-OBzl with 1 M TFMSA in TFA (0 °C; 60 min) in the presence of Me_2S (0.5 M) afforded the free form of the unsymmetrical cystine peptide amide (**1**) (yield 87%), while TFA treatment of an equimolar mixture of Z(OMe)-Cys(Acm)(O)-Ala-NH_2 and Z(OMe)-Cys(MBzl)-Gly-OBzl (25 °C; 120 min) in the presence of Me_2S afforded the unsymmetrical cystine ester (**2**) [oil, *m/z* (fast-atom bombardment): 458 $(M + H)^+$]. In confirmation of its structure the ester (**2**) was converted into the free amide (**1**) by treatment with 1 M TFMSA–0.5 M Me_2S in TFA (overall yield 71%). Small amounts of two symmetrical cystine peptide by-products were removed from the desired peptides by h.p.l.c. on a YMC R-ODS-5 column using 0.1% aqueous TFA.

This disulphide bond-forming reaction was next applied to the syntheses of oxytocin.[7] Protected oxytocin, Boc-Cys(Acm)(O)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2, prepared by the conventional solution method, was

treated (25 °C; 60 min) with TFA in the presence of Me_2S. After gel-filtration on Sephadex G-15, a highly pure product with an h.p.l.c. retention time identical with that of an authentic sample of oxytocin (Protein Research Foundation, Osaka, Japan) was obtained in 86% yield.

As demonstrated in these experiments, the disulphide bond could be formed at the sulphoxide position intramolecularly, as well as intermolecularly. Recently, we reported that cystine peptides can be obtained by oxidation with $(CF_3CO_2)_3Tl$ of S-protected cysteine peptides.[8] In addition to the methods hitherto employed for synthesis of unsymmetrical cystine peptides,[9] the present alternative disulphide bond-forming reactions may be useful for synthesis of peptides containing several disulphide bonds.

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